IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 023533/0113

In re patent application of
CORNETT *et al.*

Group Art Unit: 1648

Serial No. 09/783,580

Examiner: Scott David Priebe

Filed: February 15, 2001

For: RECOMBINANT BETA2-ADRENERGIC RECEPTOR DELIVERY AND USE IN TREATING AIRWAY AND VASCULAR DISEASES

DECLARATION UNDER 37 CFR ə 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, Lawrence E. Cornett, hereby declare:

1. I am an inventor of the captioned application. I have worked in the field of adrenergic receptor structure and function, including how adrenergic receptor gene expression is regulated in mammalian tissues that are responsive to circulating catecholamines since 1977. I have published over 32 papers in this field. Attached as Exhibit A is my curriculum vitae.

2. I have read and understood the Office Action dated June 7, 2002, and particularly the Examiner's statements on page 7 of the Office Action regarding the lack of evidence of the expression of β2 adrenergic receptor (β2AR). In support of the expression of β2AR in a human subject, I provide the following experimental data.

3. A recombinant AAV vector was prepared which included two expression cassettes: 1) the β2AR driven by a CMV promoter, and 2) green fluorescent protein (GFP) driven by a CMV promoter. The AAV vector was suspended in 0.9% saline and 0.5 milliliters was delivered into the airway of Sprague-Dawley rats using a microspray device (Penn Century Microsprayer) to produce an aerosol. Control Sprague-Dawley rats received 0.5 milliliters of 0.9% saline delivered by the microspray device.

4. Two weeks later the rats were sacrificed and lungs sectioned (30 micrometer sections) for microscopy. The lung sections were examined by fluorescence microscopy to detect GFP expression. Control rats had no observable fluorescence due to GFP. Rats treated with the recombinant AAV containing the cassettes of the β2AR under the control of a CMV promoter and the GFP under the control of a CMV promoter expressed GFP as shown in the two attached figures (Figures 1 and 2) as Attachment B. Each of the figures is a separate lung section prepared from an AAV treated rat. Cells in the lung sections expressing GFP are denoted by arrows. The expression of GFP is indicative of the expression of β2AR in the lung section preparations. Thus, the marked cells in lung section express β2AR.

5. In support of my statements directly above, I have obtained direct evidence using cultured HEK293 cells that demonstrates β2AR and GFP are co-expressed from the recombinant AAV vector. My laboratory has infected HEK293 cells with the recombinant AAV vector and four days later examined β2AR levels using radioligand assays and cyclic AMP assays. By epifluorescence microscopy, 40% of the infected HEK293 cells demonstrated GFP expression. β2AR density measured with [^{3}H]dihydroalprenolol increased 11-fold in infected cells compared to control cells that were not infected with the recombinant AAV vector. Isoproterenol-stimulated cyclic AMP levels increased 5-fold in infected cells indicated the expressed receptors functionally coupled to downstream effector protein (e.g., adenylyl cyclase). These results definitely show that both expression cassettes in the recombinant AAV vector function and indicate that GFP expression is a marker for β2AR expression in recombinant AAV vector infected cells.

6. These experiments provide data to show that β2AR is expressed in the cells of rat's lungs to which AAV vectors containing the DNA encoding β2AR are administered via the rats' airways. It is my opinion that the results of the experiment provide the evidence that β2AR administered to the airways of rats is expressed in cells of the lungs of these animals.

7. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 11/7/02

By: L. E. Cornett
Lawrence E. Cornett, Ph.D.